Evolution of DNA: Changes in Gene Regulation

Evolution of living creatures has traditionally been studied from two points of view: Taxonomists have focused on changes in morphology as animals evolved and molecular biologists have focused on changes in DNA. Changes in DNA could involve changes in genes or changes in DNA sequences involved in gene regulation. Since changes in genes are easier to detect than changes in regulatory DNA, most research in molecular evolution has stressed the evolution of genes. However, it has been difficult to reconcile changes in genes with changes in animal anatomy during evolution. Thus, many investigators are shifting their emphasis from genes to regulatory DNA in their attempts to understand how evolution occurs.

The idea that regulatory DNA may be more important than genes to the process of evolution is not new. In the past few years, proponents of this idea have included Roy Britten and Eric Davidson of the California Institute of Technology and Susumu Ohno of the City of Hope Medical Center in Duarte, California. But only recently has a substantial collection of evidence been reported in support of this hypothesis.

Changes in structural genes—that is, DNA sequences that code for proteins—are relatively easy to detect since proteins from various species can be isolated and compared by various biochemical techniques. The well-recognized species differences in proteins, such as hemoglobins, have been attributed to differences in genes coding for those proteins in the individual species.

Allan C. Wilson and his associates at the University of California at Berkeley compared blood proteins—specifically hemoglobin, albumin, and transferrin—of various vertebrates to see whether changes in structural genes are related to anatomical evolution. They reasoned that, if structural genes change as organisms evolve, then the most similar species, defined as those that can mate and produce offspring (hybridize), should have the most similar structural genes. If blood proteins are a representative sample of proteins coded by structural genes, the most similar species should have the most similar blood proteins. Wilson and his colleagues found, however, that structural genes for blood proteins accumulate mutations at rates that appear independent of anatomical evolution. Species that diverged most recently, rather than species that are most closely related, have the most similar blood proteins.

Wilson and his colleagues report that mammals that form hybrids have very similar blood proteins. However, frogs, which are a more ancient species, can hybridize even when their blood proteins are very different. For example, about 2 percent of the amino acids of albumin molecules of mammals that can hybridize are different when they are compared by the immunological technique of micro-complement fixation. This reflects changes in DNA since proteins consist of sequences of amino acids and each amino acid is coded by a linear array of three DNA nucleotides. In contrast to mammals, albumin molecules of frogs that can hybridize differ in about 20 percent of their amino acids. Mammals have existed for about 75 million years, whereas frogs have inhabited the earth for about 150 million years.

Additional evidence that changes in structural genes may not be correlated with anatomical evolution was recently reported by Mary-Claire King of the University of California at San Francisco and Wilson. They compared a group of 44 proteins of human beings and chimpanzees—two species so dissimilar that they are placed by taxonomists in different families. However, King and Wilson found that the human proteins are, on the average, 99 percent identical to those of the chimpanzees. This means that the structural genes coding for these 44 proteins are as similar as the structural genes of species classified as sibling species. Sibling species, unlike human beings and chimpanzees, are virtually identical morphologically. King and Wilson suggest that changes in gene regulation rather than changes in structural genes are the key to anatomical evolution.

Genes Evolve Slowly

The 44 structural genes studied by King and Wilson represent only a small fraction of the structural genes of a chimpanzee or a human being. Many investigators estimate that mammals may have as many as 10,000 to 30,000 structural genes. It remains possible that King and Wilson analyzed a nonrepresentative sample of structural genes and that most changes in structural genes are correlated with anatomical evolution. However Michael Rosbash of Brandeis University, together with Kenneth S. Gummerson and M. Savario Campo of the University of Edinburgh, recently obtained results indicating that structural genes of rats and mice evolve far more slowly than the remainder of the DNA, a finding that is consistent with the hypothesis that rates of change of structural genes may not reflect anatomical evolution.

Rosbash, Gummerson, and Campo used a group of structural genes of rat myoblasts (immature muscle cells) for their comparative studies. They identified these structural genes as those transcribed into messenger RNA molecules containing sequences of polyadenylate. Although there are likely to be other structural genes in rat myoblasts, these investigators assume that these genes—which code for 7000 different proteins—are representative of rat structural genes.

Rosbash, Gummerson, and Campo studied the similarity of these rat structural genes to those of mice. They compared this result to their analysis of how similar a much larger class of DNA sequences—namely, single copy DNA sequences—of rats are to analogous sequences of mice. Single copy DNA includes structural genes but also includes many DNA sequences that do not code for proteins and whose functions are unknown. Rosbash and his associates estimate that at most 20 to 30 percent of the single copy DNA of rat cells consists of structural genes.

Rosbash and his associates used the technique of molecular hybridization—a means of measuring sequence differences between DNA molecules—to determine that the structural gene sequences of rats and mice are about twice as similar as the single copy DNA sequences of those animals. About 14 percent of the nucleotides in single copy DNA of rats are different from those of mice, but only 5 to 8 percent of the nucleotides of structural genes of rats are different from those of mice.

In addition to single copy DNA, cells of higher organisms contain another class of DNA sequences—repeated DNA sequences—that were not studied by Rosbash and his associates. Each repeated sequence is present between about 10 and 5000 times per cell. Although structural genes are present among repeated DNA sequences, what fraction of the repeated sequences are structural genes is unknown. A complication in interpreting results on the evolution of DNA arises from the possibility that repeated and single copy DNA sequences may evolve in different ways. Glenn Galau, working with Britten and Davidson, found that this may occur in amphibians. He presented his results at the meeting of the Society for the Study of Evolution and the American Society of Naturalists, held on 16 to 18 June 1975 in Davis, California.

Galau and his associates compared the

single copy and repeated DNA sequences of two frog species—*Xenopus laevis* and *Xenopus mulleri*. These two species can mate and produce offspring, although the offspring are infertile. The single copy sequences of these frogs, they found, had diverged a great deal. At least 10 to 15 percent of the nucleotides of the single copy DNA of *Xenopus laevis* differs from those of *Xenopus mulleri*. In contrast, the nucleotides of the repeated DNA sequences of the two species differ by only 1 to 2 percent.

Although the nucleotides of the repeated sequences changed very little, the frequency with which a given repeated sequence occurs in cells of the two frog species apparently changed substantially when the species evolved. Groups of repeated sequences of *Xenopus laevis* DNA are present at 10 to 100 times lower frequencies in *Xenopus mulleri* DNA, Galau reports. Moreover, different groups of repeated sequences of *Xenopus mulleri* DNA are present at 10 to 100 times lower frequencies in *Xenopus laevis* DNA. As Britten and Davidson point out, these results are difficult to interpret without more knowledge of the way gene expression is regulated and the meaning of chromosome organization in higher organisms.

A lack of an explanation of how genes are regulated in higher organisms is becoming a major stumbling block in studies of molecular evolution. Although mechanisms of gene regulation are, for the most part, unknown, there is evidence that two kinds of changes in DNA can alter patterns of gene regulation: The nucleotide sequences of certain regulatory segments of DNA could be changed and the organization of genes on chromosomes could be changed.

Wilson pointed out at the meeting in Davis that examples of changes in regulatory sequences of DNA during evolution have been known for nearly a decade, although their significance was seldom recognized. Investigators showed that, in most cases, bacteria adapt to new environments by means of mutations that alter gene regulation rather than mutations that alter the sequences of structural genes.

In a typical experiment, bacteria are placed in a culture medium with a nutrient that they cannot utilize because they have no enzymes to break it down. Most of the bacteria die if this nutrient is their sole source of energy. However, a few mutants do live. Upon analysis, these mutants are found to possess alterations in a regulatory sequence of DNA that enables them to produce enormous quantities of an enzyme that they normally make in small amounts and that, by chance, has a slight ability to degrade the nutrient. The enormous quantities of this enzyme make up for its inefficiency in degrading the growth compound and the mutants live. After a time, further changes occur in the structural gene for this enzyme to make the enzyme more efficient in degrading the growth compound. But the first event is the mutation in a regulatory sequence of DNA. Such effects were demonstrated by many investigators, including Joshua Lederberg of Stanford University, E. C. C. Lin and his associates at Harvard University, and, more recently, Patricia Clarke of University College in London and her associates.

As yet, there is no evidence that gene regulation in higher organisms resembles that in bacteria. However, Wilson stresses that, since even bacteria evolve by means of changes in gene regulation, it is likely that higher organisms do also.

The other way that changes in gene regulation can occur—by alterations in chromosomal organization—is less well understood. However, Wilson and his associates report that frogs, which are anatomically simple organisms, exhibit fewer chromosomal changes when they evolve than mammals, which, of course, are more complex.

Changes in Chromosomes

Two types of changes in chromosomes can be detected microscopically: changes in number (indicating breaking, joining, and duplication of DNA segments) and changes in shape (indicating inversions or rearrangements of DNA segments). Wilson and his colleagues report greater incidences of both kinds of changes in mammals than in frogs.

Wilson interprets his results on chromosomal changes in terms of rates of evolution. He, along with other investigators, believes that frogs evolved more slowly than mammals. Thus a slow rate of anatomical evolution could be associated with few chromosomal changes. Thomas Schopf of the University of Chicago, David Raup of the University of Rochester, Stephen Jay Gould of Harvard University, and Daniel Simberloff at Florida State University, on the other hand, recently presented evidence that different species evolve at similar rates. If they are correct, Wilson's results would have a different interpretation.

Schopf and his associates note that anatomically very complex organisms have more morphological features that can change during evolution than less complex organisms. If changes in chromosome organization are associated with anatomical changes, then more complex organisms, with their greater number of possible anatomical changes, would have larger numbers of possible chromosome changes. If chromosome changes are random events that affect chromosome segments of all species equally, more complex species should exhibit more chromosome changes. With more ways to rearrange genes on chromosomes of complex organisms, it is more likely that a random event will result in a chromosome change.

The question of how changes in gene regulation can lead to anatomical changes is open to speculation. Gould, though, has resurrected the "fetalization theory," proposed in the 1920's by the Dutch anatomist Louis Bolk to explain what sort of regulatory changes could have allowed human beings and chimpanzees to evolve from a common ancestor while retaining nearly identical structural genes. Bolk wrote that "man, in his bodily development, is a primate fetus that has become sexually mature," and listed more than 20 traits that human beings share with fetal apes and monkeys to support his hypothesis. For example, people and embryonic apes and monkeys have rounded, bulbous craniums, small jaws, and an unrotated nonopposable big toe.

Although Bolk's fetalization theory never gained widespread acceptance, Gould suggests that it is essentially correct. Changes in gene regulation, he claims, retarded developmental changes by retarding the sequence of gene expression in humans more than in apes and enabled human beings and apes to evolve from a common ancestor without substantial changes in structural genes. Both Gould and Wilson point out that there are large differences in chromosome structure between human beings and other primates that conceivably could be tied to this developmental slowdown.

According to Gould, the adaptive significance of retarded development may be to permit more advanced animals a longer period to mature and thus a longer period in which to learn. Primates mature more slowly than other mammals and, among the nonhuman primates, more advanced species, such as apes, mature more slowly than less advanced species, such as monkeys.

The answer to the question of how species evolve, then, apparently involves changes in gene regulation and so awaits further studies of chromosome organization and control of gene expression in higher organisms. It thus seems evident that the old method of comparing proteins of different species may no longer be the primary tool for investigating the mechanisms underlying the evolution of organisms.—GINA BARI KOLATA